REMARKS

Status of the Claims

Claims 1, 4, 5, 9, 11-18, 20-22, 25, 30 and 31 are currently pending in the application. Claims 1, 4, 5, 9, 11-18, 20-25 stand rejected. Claims 1, 14, 16 and 18 have been amended. Claims 23 and 24 have been cancelled. All amendments and cancellations are made without prejudice or disclaimer. New claims 30 and 31 have been added. No new matter has been added by way of the present amendments. Specifically, the amendment to claim 1 is supported by the specification at, for instance, page 9, lines 10-12 (pore density of the filter in step b), page 19, lines 1-2 (filtration depressure in step b), previous claim 1, wherein in step c) the feature of analyzing the cells for the presence of at least one Immunological marker, which had been deleted in our previous response dated March 28, 2007, has been reinstated; and wherein at the end of claim 1: the purification step, which had been added in our previous response dated October 31, 2007 has been currently deleted from claim 1 to reinstate it in a dependent claim (please see new claim 31) and previous claims 23 and 24. New claim 30 is supported by the content of the application as filed, for example by page 9 lines 14-15. New claim 31 is the reinstatement of previous claim 10 (pre-amplification step). Claims 14, 16 and 18 have been amended in accordance with currently amended claim 1 and currently added claim 31. Reconsideration is respectfully requested.

Rejections Under 35 U.S.C. § 103(a)

Claims 1, 4, 5, 9, 11, 12, 20-25 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Kalionis in view of Vona. (*See*, Office Action of February 5, 2008, at pages 2-9, hereinafter, "Office Action").

Claims 13, 14, 16 and 17 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Kalionis in view of Vona and further in view of Bianchi. (*See*, *Id.* at pages 9-11).

Claim 15 stands rejected under 35 U.S,C. § 103(a) as being unpatentable over Kalionis in view of Vona and Fodor. (*Id.* at pages 11-12).

Claim 18 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Kalionis in view of Vona, and further in view of Pinkel. (*Id.* at pages 12-13).

Claims 23 and 24 have been cancelled herein without prejudice or disclaimer, thus obviating the rejection of these claims. Applicants traverse these rejections as to the remaining claims, and as set forth in their replies of November 28, 2006, of March 28, 2007 and of October 31, 2007, the entireties of which are incorporated herein by reference as if each and every statement were represented herein, to address each and every rejection listed above.

More specifically, Applicants maintain that neither Kalionis nor Vona, nor any of the other references, disclose a step which resembles step b) of claim 1.

The method of Kalionis comprises:

- filtering a sample of the maternal blood,
- collecting all the cells that are retained on the filter, and then,
- submitting all the collected cells to immunostaining with trophoblast-reactive antibodies, to identify the trophoblast cells, and then

- submitting the cells to in situ hybridization.

Contrary to step c) of the presently claimed invention, the Kalionis reference does not disclose and does not teach collecting one individual cell from the filter. In the Kalionis reference, there is no step wherein a cell, which would be presumed of being of a fetal origin, would be isolated.

Contrary to step c) of the presently claimed invention, the Kalionis reference does not disclose and does not teach a step which comprises analyzing the cells that are retained on the filter before individually collecting an appropriate cell therefrom, i.e., a cell, the (fetal) origin of which would be presumed.

Therefore, the Kalionis reference does not disclose a step that resembles step c) of the presently claimed method

Hence, both the nature and the order of the method steps of the Kalionis reference differ from that recited in present claim 1.

It should also be duly taken into account that, in accordance with step f) of the presently claimed invention, the demonstration of fetal origin, and the prenatal diagnosis, are both performed on one single cell. More precisely, these steps are performed on the pre-amplified genome of one single trophoblastic and/or syncytiotrophoblastic cell.

The Kalionis reference does not disclose and does not teach any step that resembles step f) of the presently claimed invention, i.e., a step wherein a dual genetic analysis is performed (to confirm the fetal origin of the cell and to detect a genetic or chromosomic anomaly).

It is furthermore maintained that the method of Kalionis is suitable for late stage gestation, but not for early stage, contrary to the presently claimed invention (5 weeks of

gestation), which implies that the method of Kalionis is not enabled for pre-natal diagnosis.

Indeed, as the Kalionis method is not performed on an isolated fetal cell (nor on isolated fetal cells), but on a mixed cell population, which is enriched in fetal cells (trophoblast cells), but which still comprises other cell types (including maternal cells), the Kalionis method is only enabled for pregnant women, who are at the end of gestation, e.g., in their 30-37th week of gestation, i.e., 7.5 months of gestation and over (see Table 1 of the Kalionis reference, at page 21).

To the contrary, the method of claim 1 of the present application is enabled for pregnant women at any stage of pregnancy, and more particularly at the very early stages of pregnancy, e.g., as early as 5 weeks of gestation (see page 7 lines 15 and 18 of the present application, as well as claim 20).

The method of the presently claimed invention is precisely suited to the early stage of pregnancy. The presently claimed invention is precisely meant to address this problem.

Hence, Applicants maintain that, starting from the Kalionis reference, and whichever document is combined therewith, one of ordinary skill in the art has to change everything in the Kalionis disclosure to arrive at the presently claimed invention, not just certain elements.

Applicants also maintain that the Kalionis reference is not enabled to address the problem of pre-natal diagnosis.

In the Office Action, the document with which the Kalionis document is primarily combined is the Vona reference, which relates to a method of Isolation by Size of Epithelial Tumors cells (ISET method). In the Vona reference, there is only one single assay comprising both ISET and RT-PCR. This (ISET + RT-PCR) assay is performed on an epithelial tumor cell

line, namely the Hep3B cell line.

In this assay, all the cells that are retained on the ISET filter are tumor cells, whereas, in the presently claimed method, the cells that are retained on the filter are a complex population of cells comprising a mixture of different cell types, such as fetal and maternal cells.

In the Vona reference, the starting cell population is a very simple one: it is a pure population of tumor cells from a tumor cell line. Therefore, the Vona reference does not address the problem of identifying rare cells within a larger and complex cell population.

In contrast, the presently claimed invention solves the problem of the isolation of circulating fetal cells, i.e., of cells that are very rare and that are contained *in vivo* in mixture with many other cell types. The starting material is a very complex population of many different types of cells, which include maternal epithelial cells and leukocytes.

Hence, there is a huge technical gap between the situation faced in the Vona reference and the one faced and solved by the present inventor.

As a matter of fact, the Vona reference does not disclose a step which would comprise analyzing the cells that are retained on the filter before individually collecting an appropriate cell therefrom, i.e., a cell, the (fetal) origin of which would be presumed.

Therefore, the Vona reference does not disclose and does not teach a step that would resemble step c) of the presently claimed method. Therefore, the Vona reference does not overcome the deficiencies of the Kalionis reference in this respect.

Furthermore, contrary to step f) of the presently claimed invention, the Vona reference does not disclose a method wherein the amplification step would comprise the demonstration of two clinical features, whereas in the claimed method of the present invention, the amplification

step is intended for both demonstrating the fetal origin of the collected cells and for carrying the prenatal diagnosis as such and both demonstrations are performed on the genome of one single cell (see step f) of the claimed method.

Therefore, neither does the Vona reference overcome the deficiency of the Kalionis reference in this respect.

Furthermore, whereas the Vona reference suggests testing whether ISET would be applicable to the filtration of trophoblast cells, it nevertheless does not suggest applying (ISET + RT-PCR) or (ISET + PCR), or more precisely (ISET + immunological/cytological analysis + PCR), to trophoblast cells. Therefore, the Vona reference does not disclose and does not suggest a method comprising both steps c) and f) of the claimed invention.

Once again, the Vona reference does not overcome the deficiency of the Kalionis reference in this respect.

None of the other references cited by the Examiner, including the Bianchi, Fodor and Pinkel references, overcome any of the various deficiencies of the Kalionis and/or Vona reference(s) described above.

It is more particularly submitted that neither Kalionis nor Vona, and none of the other cited references, including the Bianchi, Fodor and Pinkel references, teach or suggest step c) of the presently claimed method.

It is more particularly submitted that neither Kalionis nor Vona, and none of the other cited references, including the Bianchi, Fodor and Pinkel references, teach or suggest step f) of the presently claimed method.

It is more particularly submitted that neither Kalionis nor Vona, and none of the other

cited references, including the Bianchi, Fodor and Pinkel references, teach or suggest step c) and step f) of the claimed method.

Furthermore, in addition to the comments presented in our replies of November 28, 2006, March 28, 2007, and October 31, 2007, and although Applicants do not agree that the claims that were then on file were obvious in light of the cited references, Applicants have presently amended claim 1 to expedite prosecution, but without prejudice or disclaimer.

Current claim 1 recites that the claimed method involves the filtration:

- on a filter, which has a pore size of 8 μm and a pore density in the range 5×10^4 to 5×10^5 pores/cm^2, and under an applied filtration depressure, said filtration depressure in the range of 0.05 to 0.8 bars;

Referring now to step b) of present claim 1, none of the cited references, more specifically none of the Kalionis, Vona, Bianchi, Fodor and Pinkel references, disclose the filtration step of the presently claimed invention. Therefore, whichever reference combination is made, a person of ordinary skill in the art cannot arrive at the presently claimed invention.

It is more particularly submitted that, as previously mentioned, the claimed invention relates to a very specific problem, i.e., the problem of pre-natal diagnosis, and that it involves trophoblast cells, i.e., cells that are very rare in the maternal blood. The presently claimed invention cannot be equated to any standard kind of cell analysis.

With the purpose of addressing the specific problem of the isolation of said very rare circulating fetal cells, in such a way as to enable a reliable pre-natal diagnosis, the inventor has determined which parameters were most critical, which had to be the most carefully chosen, and at which most preferable value ranges these critical parameters should be adjusted.

These critical parameters notably include the choice of a filter with a particular pore density, and the application of a particular depressure range.

The Kalionis reference does not disclose nor suggest using a filter which has a pore size of 8 μm and a pore density in the range 5×10^4 to 5×10^5 pores/cm^2, and does not disclose or suggest applying any kind of filtration pressure or depressure.

The Kalionis reference does not suggest that the filter pore density and/or the pressure/depressure would be important. The Kalionis reference is simply silent about these two parameters.

Turning now to the Vona reference, if the reader has foreknowledge of the presently claimed invention, they can point out that the Vona reference discloses the use of "a polycarbonate Track-Etch-type membrane (Cyclotron Technology) with calibrated, 8-μm-diameter, cylindrical pores", and to apply "gentle aspiration under vacuum (created by a vaccum pump)." However, it must at the very least be acknowledged the Vona reference does not disclose a 5×10^4 to 5×18^5 pore density range, and does not disclose a 0.05-0.8 bar range.

Most importantly, it is only when the reader has foreknowledge of the presently claimed invention that these two passages of the Vona reference can be selected among all the other parameters disclosed in said reference.

When the Vona reference is read without foreknowledge of the presently claimed invention, it should be recognized that this reference contains no indication which would specifically direct a person of ordinary skill in the art toward the presently claimed pore density and depressure parameters rather than toward any other of the various parameters of the method disclosed in the Vona reference.

Furthermore, as a person of ordinary skill in the art is arguably taking the Kalionis reference into account, this person would then not place any criticality on the pore density of the filter and not associate any criticality with the pressure/depressure (if any) that is applied for filtration, because the Kalionis reference, which, *arguendo*, may disclose a pre-natal diagnosis method, is completely silent with respect to these two parameters. Therefore, these two parameters would appear to be of no importance at all to a person of ordinary skill in the art having read these two references.

As a matter of fact, there is no well-founded reason for a person of ordinary skill in the art to select the pore density parameter and the depressure parameter, among the various other potential parameters, of the method disclosed in the prior art references, more particularly in the Vona reference.

Asserting that the person of ordinary skill in the art would somehow "adjust" these two parameters, whereas there are many other parameters that could potentially be "adjusted," would amount to an *ex post facto* reconstruction of the prior art references, more particularly of the Vona reference.

Moreover, at least at the time when the present invention was made by the inventor, for each filter with a given pore diameter, very different pore density ranges were available, including pore densities that were outside of the 5×10^4 to 5×10^5 range. Therefore, a filter with calibrated, 8 μm-diameter, cylindrical pores (as it is disclosed in the Vona reference) does not necessarily have a pore density of 5×10^4 to 5×10^5 pores/cm^2.

Furthermore, contrary to the Examiner's assertion made at page 9 of the Office Action, the Vona reference does not disclose and does not suggest the criticality of selecting a filter wherein the pores are spaced apart to allow the separation and collection of individual cells.

It is respectfully submitted that the complexity of the problem addressed by the presently claimed invention has been underestimated by the Examiner. The problem solved by the present invention does not simply amount to the separation and collection of individual cells; rather, it relates, *inter alia*, to the isolation of very rare circulating cells present in a very complex mixture of cells.

Regarding the depressure feature of the presently claimed invention, it should be acknowledged that a vacuum pump (as it is disclosed in the Vona reference) is not capable of applying a depressure of 0.05-0.8 bar.

If a person of ordinary skill in the art were trying to use the Vona method, which in the Vona reference is disclosed to be adapted to the isolation of tumor cells from a pure culture of tumor cells, and to apply it to achieve another and very different result, i.e., the isolation of the very rare circulating fetal cells, this person would have no indication or direction anywhere in the cited references as to the solution of somehow "adjusting" the filter pore density and the filtration depressure value.

The other prior art references, including the Bianchi, Fodor and Pinkel references, do not overcome the deficiencies of the Kalionis and/or Vona reference(s) as just described. Without foreknowledge of the presently claimed invention, a person of ordinary skill in the art would be at a loss in determining which one(s) of the myriad potential parameters should be carefully set

to achieve the isolation of the very rare circulating fetal cells, and at which values these critical parameters should be set.

As a matter of fact, the identification of the criticality of these parameters was not immediate to even the present inventor of the presently claimed invention, when she tried to achieve the isolation of the very rare circulating fetal cells,

None of the cited references disclose or suggest that the pore density of the filter and the depressure value would be a critical issue to address the problem of the isolation of the very rare circulating fetal cells. To the contrary, the Kalionis reference suggests to one of ordinary skill in the art that these two parameters are of no importance.

It is therefore submitted that whichever combination is made of the cited references, many technical features, including steps b), c), and f) of the presently claimed invention, remain undisclosed and are not even suggested as being critical and/or important by any of the cited references.

Thus, it is respectfully submitted that the Examiner has failed to establish a *prima facie* case of obviousness and that the claims satsify the requirements of 35 U.S.C. § 103(a).

Furthermore, the amendments of the claims presented herein are believed to adequately address all of the Examiner's unresolved comments, if any, presented in the Official Action of February 5, 2008. That is, it is believed that the references, either considered individually or in combination, do not disclose or suggest all of the limitations recited in the presently amended claims. Thus, the Examiner has failed to establish a *prima face* case of obviousness with respect to the presently amended claims.

To establish a *prima facie* case of obviousness, there must be a reasonable expectation of success, which is not present in the Examiner's analysis, whichever combination of prior art references is made. The prior art references (or the references when combined) must teach or suggest all the claim limitations. (*See, In re Vaeck*, 347 F.2d 488 at 493, 20 U.S.P.Q.2d 1438, 1442 (Fed. Or. 1991.)).

Therefore, reconsideration and withdrawal of the obviousness rejections are respectfully requested.

CONCLUSION

If the Examiner has any questions or comments that may assist in prosecution of the present application, please contact Thomas J. Siepmann, Ph.D., Registration No. 57,374, at the offices of Birch, Kolasch & Birch, LLP.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37.C.F.R. §§1.16 or 1.14; particularly, extension of time fees.

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Respectfully submitted,

By _____

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